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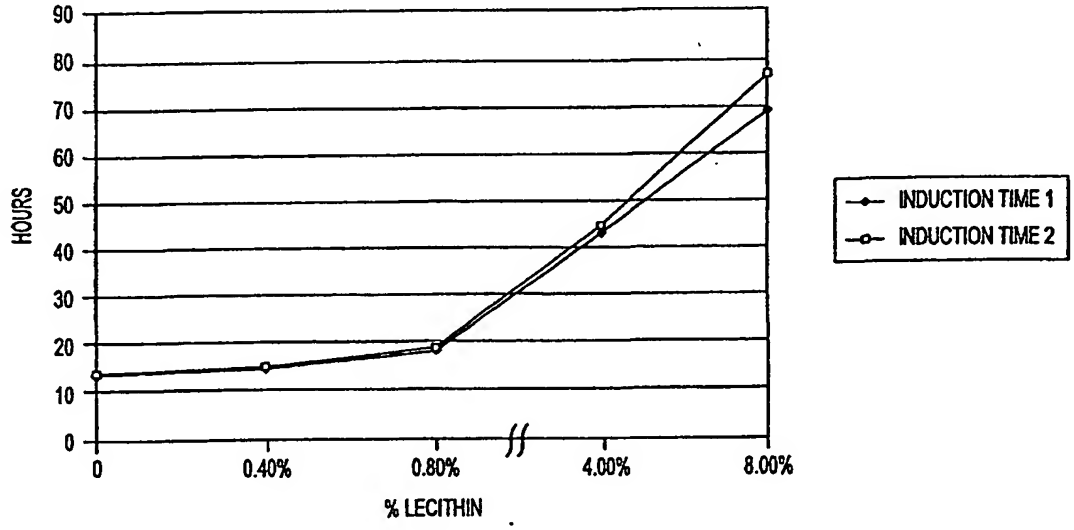

(57) Abstract

A food product containing mixed glycerides, including polyunsaturated fatty acid residues, highly unsaturated fatty acid residues, or a combination thereof, is stabilized by the addition of phospholipids. The addition of phospholipids to fat blends has been found to have an unexpected stabilizing effect on the fats contained in the blend. The phospholipids are preferably derived from vegetables, milk, microbes, or a combination thereof.

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INFANT FORMULAS AND OTHER FOOD PRODUCTS CONTAINING PHOSPHOLIPIDS

BACKGROUND OF THE INVENTION

Field of the Invention:

This invention is directed to the formulation of food products containing highly unsaturated fatty acids, and to processes for stabilizing such products.

Review of Related Art:

The importance of highly unsaturated fatty acids (HUFA) in the diet of humans, and particularly infants, is now well established (see e.g., WO 96/40106). Typical dietary sources of HUFA are organ meats, fish, eggs and human breast milk. However, present day diets are frequently deficient in HUFA, resulting in a need to supplement the diet with a source of HUFA. Sources of HUFA supplements include egg yolk phospholipids, and triglyceride oils extracted from fish and marine microorganisms. Use of egg yolk phospholipids and/or marine triglyceride oils to supply HUFA in infant formula is taught in U.S. Patent No. 4, 670,285 by Clandinin, et al., and WO 96/10922 by Milupa. Use of microbial triglyceride oils to supply HUFA in infant formula is taught in U.S. Patent Nos. 5,374,657, 5,397,591 and 5,550,156 by Kyle, et al. Another source to supply HUFA in infant formula is a lipid extract from human placenta, taught in European Patent No. 0 140 805 to Bio-Extraction.

The Clandinin and Milupa patents (use of egg yolk phospholipids) and Bio-extraction (use of placental phospholipids) all describe animal sources of phospholipids that are used in infant formulas because of their DHA and ARA contents. Phospholipids (gums) are already added to foods (including infant formula), and these phospholipids are generally vegetable-derived phospholipids because they are relatively inexpensive. However, the levels of phospholipid added are generally less than 0.5% by weight of the fat blend, especially in infant formulas, and they are added to improve the physical properties of the product. For example, phospholipids may be added as emulsifiers or wetting agents.

Because of their degree of unsaturation, HUFA are prone to oxidative degradation. Preserving the double bonds of the HUFA through processing and storage is a critical issue in the preparation and distribution of infant formula, baby food and other nutritional supplements containing such materials. European Patent No. 0 404 058 to Milupa describes the addition of alpha-tocopherol and/or ascorbyl-palmitate as antioxidants during preparation of HUFA-containing mixtures to reduce oxidative degradation. The HUFA-containing materials are added to a mixture containing antioxidants in amounts to give final concentration of from 150-300 ppm, and the mixture typically contains mono- and diglyceride emulsifiers. U.S. Patent No. 5,855,944 by Koschinski, et al., describes a process for stabilizing HUFA-containing marine oils by treating the oils with silica, steam deodorizing the oil, and then adding to the oil a mixture of food-grade lecithin, alpha-tocopherol, and ascorbyl-palmitate in a total amount of 1000-4000 ppm of the mixture.

However, there remains a need for a inexpensive, effective method for chemically stabilizing products which contain PUFAs and/or HUFAs.

SUMMARY OF THE INVENTION

In one embodiment, this invention provides a method for preparing a food product containing triglyceride oil having highly unsaturated fatty acid residues in which oxidation of the highly unsaturated fatty acid residues is minimized during the preparation by blending phospholipids with the triglyceride oil in an amount effective to reduce oxidation of the highly unsaturated fatty acid residues during subsequent processing steps and during storage. The phospholipids may be derived from vegetable sources, milk fat, milk processing waste products, microbial sources, such as yeast brewing waste products, and like sources. Preferably, the phospholipids are vegetable-derived. Preferably, the phospholipids are not derived from animal sources other than milk sources. In another embodiment, this invention provides food products containing triglyceride oil having highly unsaturated fatty acid residues, where the highly unsaturated fatty acid (HUFA) residues are partially stabilized against oxidation during processing and storage by phospholipids which are present in the food product in an amount effective to reduce oxidative damage to

the highly unsaturated fatty acid residues. Preferably, the phospholipids are obtained from vegetable sources; but they may also be obtained from milk fat, milk processing waste products, and like sources.

Food products according to this invention include infant formula and baby food. Food products according to this invention also include nutritional supplements, such as HUFA-containing oil and /or HUFA-containing biomass formulated for consumption in, e.g., capsules, tablets, emulsions (including water in oil emulsions and oil in water emulsions), or sustained release capsules. Other non-exhaustive examples of food products according to this invention include butters, spreads, cooking oils, salad dressings, and chocolate, in which part of the oil has been replaced by a HUFA-containing oil. In each of these food products, the HUFA residues are partially stabilized against oxidation by phospholipids that are present in the food product in an amount effective to reduce oxidative damage to the HUFA residues during processing and storage. Suitable methods for the preparation of these food products are well-known in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Graphic representation if the effect of lecithin on induction time of an oil blend of ARASCO® and DHASCO®.

Figure 2. Graphic representation if the effect of lecithin on induction time of an oil blend of DHASCO®.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention involves the addition of a quantity of phospholipids to the fat blend of an infant formula or other food product at levels of up to 30% by weight of the fat blend, which results in the stabilization, especially the oxidative or chemical stabilization, of that fat blend to processing steps such as, but not limited to, spray drying, retort sterilization, pasteurization, ultra high temperature (UHT) processing, or extrusion and in the stabilization of that fat blend during storage. These phospholipids are preferably sourced from vegetable material including, but not limited to, soy, corn, palm, canola, rice, flax, coconut, and combinations thereof,

and are usually obtained as a byproduct of the process of refining the vegetable oil. These phospholipids may also be obtained from milk fats, milk processing waste products, microbial sources, such as yeast brewing waste products, and like sources. Preferably, the phospholipids are not derived from animal sources other than milk sources. For the purposes of this invention, the term "non-animal" will not encompass milk or milk products or products derived from milk or milk products. These phospholipids may be comprised of any of phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE) and/or phosphatidyl inositol (PI), or a combination thereof. Unlike egg yolk phospholipids and placental phospholipids, for example, these materials are distinguished from animal phospholipids in that they are less likely to contain certain fatty acids such as docosahexaenoic acid (DHA) or arachidonic acid (ARA).

The inventors have discovered that addition of phospholipids, particularly vegetable phospholipids, has an effect of stabilizing a fat blend containing HUFAs and/or long chain polyunsaturated fatty acids (LCPUFAs), such as DHA and ARA, in an emulsion which is protected from damage, especially oxidative damage, during harsh processing steps such as, but not limited to, spray drying. Furthermore, the addition of phospholipids according to the present invention will further stabilize the final product to provide a longer shelf life. In particular, laboratory data demonstrates that the addition of soy lecithin (primarily PC) at levels of 0.1% and 0.5% tremendously reduced the oxidizability of an oil rich in ARA (e.g., ARASCO® brand (Martek Biosciences Corp., Columbia, MD, USA 21045) triglyceride oil from *Mortierella alpina*). This stabilization, unexpectedly appeared to be about 4-fold better than the addition of Vitamin E at levels of 50 or 150 ppm. Phospholipids from milk and microbial sources also have this effect.

Similarly, laboratory data demonstrates that the addition of lecithin (Yelkin TS from ADM (Archer Daniels Midland Company, Decatur, IL 62526); Yelkin contains approximately 63% phospholipids) at concentrations of from 0.4 to 8.0% to a blend of DHA-rich oil (e.g., DHASCO® brand (Martek Biosciences Corp., Columbia, MD, USA 21045) triglyceride oil from *Crypthecodinium cohnii*) and ARA-rich oil (e.g. ARASCO® brand triglyceride oil from *Mortierella alpina*)

dramatically retarded oxidation of the oil. Addition of lecithin extended the induction time in a dose-dependent manner. Induction time is a measurement of oxidative stability: the longer the induction time for a given sample, the greater its oxidative stability. Surprisingly, for every one percent lecithin added (after the first 0.4%), the induction time determined using Rancimat® (Metrohm Ltd., CH-9101, Herisau, Switzerland) was increased by approximately eight hours. Expressed in phospholipids, for every one percent of phospholipid added, the induction time was increased by ten to eleven hours. See Example 1, Table 1 and Figure 1, below.

This invention provides for the addition of a stabilizing agent of vegetable or microbial origin to an infant formula or other dietetic products. This agent is not of animal origin and products according to this invention may therefore be fully vegetarian or vegan products. Furthermore, it provides a source of phospholipids in large quantity to improve the nutritional quality of the infant formula. Of particular importance is the provision of PS, PE, and PC to the formula as these nutrients are known to be important for brain development and are found in human milk.

At present, animal phospholipids which contain DHA and ARA (e.g., egg yolk, placental lipids; see, e.g., Clandinin and Milupa patents; placental lipids patent of Bio-extraction) are being added to infant formulas as sources of DHA and/or ARA, but these sources are highly sensitive to oxidation. The present invention solves the problem of oxidation by adding large amounts of plant-, milk, and/or microbial-derived phospholipid which is not as sensitive to oxidation and which also imparts an unexpected oxidative stability to fats which contain high levels of DHA and ARA. The plant-, milk, and/or microbial-derived phospholipids may also be added to formulas where animal phospholipids such as egg yolk are already added.

Phospholipids derived from vegetable sources, milk fats, milk processing waste products, microbial sources, such as yeast brewing waste products, and like sources are not as sensitive to oxidation as other animal phospholipids that contain DHA and ARA and impart an unexpected oxidative stability to fats which contain high levels of DHA and ARA. Plant-derived phospholipids, milk-derived, and microbial-derived phospholipids may be blended together in any suitable combination in embodiments according to the present invention.

The prior art suggested that egg phospholipids or brain phospholipids should be added to infant formulas to provide sources of DHA and ARA. Providing a source of phospholipids which specifically does not contain DHA or ARA is contrary to the aforementioned inventions. Indeed, the previous patents teach away from the use of plant-derived or milk-derived phospholipids because of the deficiency of DHA and ARA in the plant phospholipids and non-human milk phospholipids. Similarly, the previous patents teach away from the use of microbial-derived phospholipids that do not contain ARA and/or DHA. Rather, the phospholipids are added to match more closely the fat blend of human milk with respect to overall complex lipids (i.e., phospholipid) content.

Lecithin (PC) at a level of from 200-2000 ppm in combination with vitamin E and ascorbyl palmitate, has been shown by Hoffman-La Roche (see product Ronoxan, an antioxidant) to have an improvement in antioxidant capability compared to each of the individual components alone. However, the benefit provided by the levels of phospholipids taught by the present invention greatly exceed the benefit provided by Ronoxan. Significantly, the benefits of adding concentrations of phospholipids higher than 2000 ppm, could not be predicted from the benefits of adding phospholipids at 2000 ppm or less.

That the present inventors' discovery is unexpected is further accentuated by the observation that in Ronoxan the combination of ascorbyl palmitate and tocopherol is required in conjunction with phospholipids in order to provide antioxidant capability. The methods and products of the present invention have no such requirement. As shown in Example 2, which compares induction times for DHASCO® plus 250 ppm ascorbyl palmitate and 250 ppm mixed tocopherols supplemented with the phospholipids of the present invention vs. DHASCO® supplemented only with the phospholipids of the present invention, a given amount of phospholipids is effective to substantially increase the stability of the oil for DHASCO® without additional antioxidants as well as for DHASCO® with additional antioxidants.

The present invention provides for the addition of vegetable-derived, milk-derived, and/or microbial-derived phospholipids to the fat blend or food fat of infant

formulas and other food products at levels up to 30% by weight of the fat blend, formula fat, or other food fat. Preferably, the phospholipids are added at levels of from 0.4% to 20% (preferably 0.5% to 20%, more preferably 1% to 16%, and even more preferably 4% to 16%) of the fat blend. Preferably the phospholipids are added in an amount effective to substantially increase the stability, particularly the oxidative stability, of the fat blend. An amount effective to substantially increase the stability is an amount effective to increase the induction time by 50% above the induction time of the same fat blend to which the phospholipids of this invention have not been added. Preferably, the effective amount of phospholipids will increase the induction time by a factor of 2 to 5 times greater than the induction time of the same fat blend to which the phospholipids of this invention have not been added.

Typically, greater increases in induction time are preferable. However, it is understood that increasing the amount of phospholipids added to a fat blend above a certain amount may not be desirable in certain contexts or conditions. Therefore, it is understood that a balance may be found wherein the stability of the fat blend is substantially increased, but phospholipids are not added to an undesirable level.

The amount of the phospholipids of the present invention which will constitute an effective amount may vary with the components of the fat blend the stability of which is desired to be increased. The optimal amount of phospholipids to add to a particular fat blend may be determined through routine optimization studies. As an example, Example 1 presents an experiment wherein the stabilizing effect of the phospholipids of the present invention on a blend of DHASCO® and ARASCO® is examined. In contrast, Example 2 an experiment wherein the stabilizing effect of the phospholipids of the present invention on DHASCO® is examined. It can be seen from the results that optimum amounts of phospholipids vary slightly between the blend versus the DHASCO® alone.

The phospholipids of this invention may be added in addition to other stabilizing agents such as, but not limited to, tocopherols, ascorbic acid and its derivatives, such as ascorbyl palmitate, and BHT. Such stabilizing agents will be referred to as "additional stabilizing agents" or "additional antioxidants."

The high levels of phospholipids conceived in this invention provide unexpected chemical (e.g., oxidative) stability to the fat blend used in the formula or food by generating an emulsion which is beneficial to the spray drying process. The levels of phospholipids added according to the present invention are much higher than the upper limits previously though to be useful, and it is an unexpected result that the increasing levels of phospholipid addition provide increasing levels of stability. Further, the phospholipids of the present invention provide their stabilizing effect in the absence of any other antioxidants or stabilizers. However, such other antioxidants and/or stabilizers may optionally be added to oil blends in combination with the phospholipids of the present invention.

Furthermore, the addition of phospholipids should impart an additional nutritive benefit to the infant formula as these components are important in the metabolism and physiology of the growing infant. Furthermore, the addition of the phospholipids have imparted a previously unexpected stability on certain oils which are rich in ARA and /or DHA, such as, but not limited to, ARA-containing oil from *Mortierella alpina* (see U.S. Patent No. 5,658,767 to Kyle), DHA-containing oil from *Crypthecodinium cohnii* (see U.S. Patent No. 5,492,938 to Kyle et al.; U.S. Patent No. 5,407,957 to Kyle, et al.; U.S. Patent No. 5,397,591 to Kyle et al.), and DHA-containing oil from *Thraustochytrium* species (see U.S. Patent No. 5,130,242 to Barclay).

Preparation of the source HUFA-containing oils and manufacture of HUFA-containing foods and nutritional supplements, including infant formula and baby food, are described in more detail in the various patents and patent applications cited herein, which are all incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Example 1.

The antioxidant effectiveness of lecithin in a blend of DHASCO® (a DHA-rich oil from *Crypthecodinium cohnii*) and ARASCO® (an ARA-rich triglyceride

oil from *Mortierella alpina*) was investigated. DHASCO® and ARASCO® each are supplemented with 250 ppm ascorbyl palmitate and 250 ppm mixed tocopherols.

Lots of Formulaid (an oil containing ARASCO® and DHASCO®) were blended to yield an oil containing DHA and ARA in a ratio of 1:1.5. Specifically, lots F01723-DS-7 (DHA:ARA of 1:1) and F01723-DS-1 (DHA:ARA of 1:2) were blended in a 1:1 ratio.

Bleached lecithin (Yelkin TS from ADM, lot #PG386) was added to the oil blend. The lecithin contained approximately 63% phospholipids (as acetone insoluble matter) and soybean oil. ADM states that the lecithin naturally contains approximately 1000 ppm tocopherol, but no added antioxidants. The lecithin was added to the oil blend at concentrations of 0.4%, 0.8%, 4.0%, and 8.0% and mixed well. Induction time was analyzed on a Rancimat using standard settings (20 mL/minute, 90°C) according to the Official Method set forth by the American Oil Chemists' Society (AOCS) (see, Official Methods and Recommended Practice of the AOCS, 5th ed., Cd12.b-92, "Oil Stability Index"). Oxidative stability may also be assessed using the active oxygen method (AOM), which is well-known in the art.

Addition of lecithin extended the induction time in a dose-dependent manner. Surprisingly, for every one percent Yelkin added, the induction time was increased by approximately seven to eight hours. Expressed in phospholipids, for every one percent of phospholipid added, the induction time was increased by approximately ten to twelve hours. These effects are much greater than can be explained by any effects of dilution of the oil blend. Table 1 presents the results in tabular form; Figure 1 presents results in graphic from.

Example 2.

The antioxidant effectiveness of lecithin in DHASCO® (a DHA-rich oil from *Crypthecodinium cohnii*) supplemented with 250 ppm ascorbyl palmitate and 250 ppm mixed tocopherols was investigated and compared to the antioxidant

effectiveness of lecithin in DHASCO® with neither ascorbyl palmitate nor tocopherols added ("DHASCO® w/o antioxidant").

Bleached lecithin (Yelkin TS from ADM, lot #PG386) was added to the DHASCO®. The lecithin contained approximately 63% phospholipids (as acetone insoluble matter) and soybean oil. ADM states that the lecithin naturally contains approximately 1000 ppm tocopherol, but no added antioxidants. The lecithin was added to the DHASCO® at concentrations of 1.0%, 2.0%, 4.0%, and 8.0% or 16.0% and mixed well. Induction time was analyzed on a Rancimat using standard settings (20 mL/minute, 90°C) according to the Official Method set forth by the American Oil Chemists' Society (AOCS) (see, Official Methods and Recommended Practice of the AOCS, 5th ed., Cd12.b-92, "Oil Stability Index"). Oxidative stability may also be assessed using the active oxygen method (AOM), which is well-known in the art.

Addition of lecithin extended the induction time in a dose-dependent manner. Surprisingly, for every one percent Yelkin added, the induction time was increased by approximately four to five hours. Expressed in phospholipids, for every one percent of phospholipid added, the induction time was increased by approximately seven to eight hours. These effects are much greater than can be explained by any effects of dilution of the oil. Table 2 presents the results in tabular form; Figure 2 presents results in graphic from.

For purposes of clarity of understanding, the foregoing invention has been described in some detail by way of illustrations and examples in conjunction with specific embodiments, although other aspects, advantages and modifications will be apparent to those skilled in the art to which the invention pertains. The foregoing description and examples are intended to illustrate, but not limit the scope of the invention. Modifications of the above-described modes for carrying out the invention that are apparent to persons of skill in food science, agricultural engineering, edible oil processing, and/or related fields are intended to be within the scope of the invention, which is limited only by the appended claims. All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains.

Table 1

Lecithin Concentration	Phospholipid Concentration	Induction Time 1 (in hours)	Induction Time 2 (in hours)
No addition	No addition	13.7	14.0
0.4%	0.252%	14.4	15.0
0.8%	0.504%	17.9	18.4
4.0%	2.52%	42.6	44.3
8.0%	5.04%	68.8	76.4

Table 2

Lecithin Concentration	Phospholipid Concentration	DHASCO: Induction Time (in hours)	DHASCO w/o antioxidant: Induction Time (in hours)
No addition	No addition	16.4	3.5
1.0%	0.63%	13.5	10.2
2.0%	1.26%	15.9	15.8
4.0%	2.52%	22.8	24.3
8.0%	5.04%	47.7	
16.0%	10.08%		83.9

CLAIMS:

1. A food product containing mixed glycerides, wherein the mixed glycerides comprise polyunsaturated fatty acid residues, highly unsaturated fatty acid residues, or a combination thereof, and further wherein the polyunsaturated fatty acid residues, highly unsaturated fatty acid residues, or combination thereof is stabilized by the addition of phospholipids.

2. The food product according to claim 1, further wherein the phospholipids are derived from non-animal sources.

3. The food product according to claim 1 wherein the phospholipids are derived from vegetable, milk, or microbial sources, or a combination thereof.

4. The food product according to claim 1, further wherein the phospholipids are derived from byproducts of the process of refining vegetable oil.

5. The food product according to claim 1, further wherein the phospholipids are derived from milk processing waste products.

6. The food product according to claim 1, further wherein the phospholipids are derived from yeast brewing waste products.

7. The food product of claim 1, further wherein the food product is an infant formula or baby food.

8. The food product of claim 1, further wherein the food product is a nutritional supplement.

9. The food product of claim 1 further comprising additional antioxidants.

10. The food product of claim 1, wherein additional antioxidants are not added to the food product.

11. A method of preparing a food product containing mixed glycerides, wherein the mixed glycerides comprise polyunsaturated fatty acid residues, highly unsaturated fatty acid residues, or a combination thereof, comprising blending phospholipids with the mixed glycerides such that the polyunsaturated fatty acid

residues, highly unsaturated fatty acid residues, or combination thereof is stabilized by the addition of the phospholipids.

12. The method according to claim 11, further wherein the phospholipids are derived from non-animal sources.

13. The method according to claim 11 wherein the phospholipids are derived from vegetable, milk, or microbial sources, or a combination thereof.

14. The method according to claim 11, further wherein the phospholipids are derived from byproducts of the process of refining vegetable oil.

15. The method according to claim 11, further wherein the phospholipids are derived from milk processing waste products.

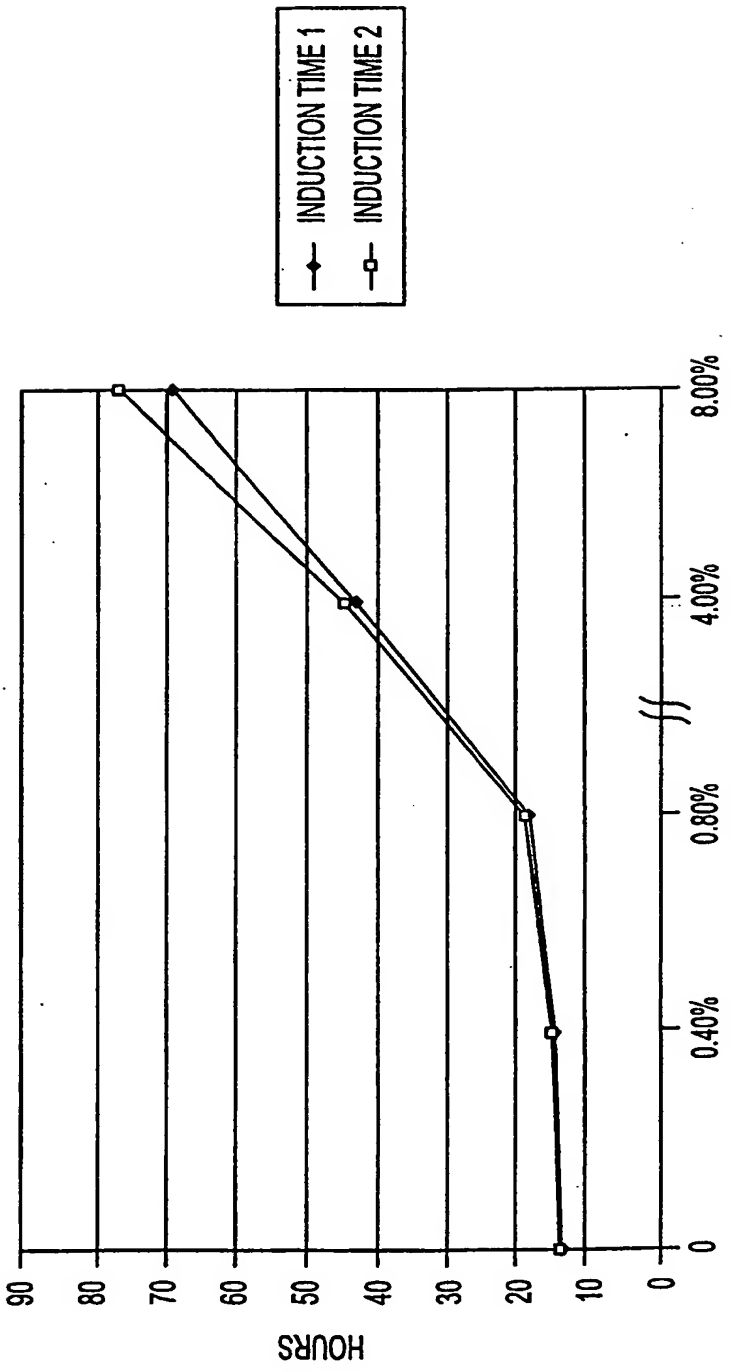
16. The method according to claim 11, further wherein the phospholipids are derived from yeast brewing waste products.

17. The method of claim 11, further wherein the food product is an infant formula or baby food.

18. The method of claim 11, further wherein the food product is a nutritional supplement.

19. The method of claim 11 further comprising adding additional antioxidants to the food product.

20. The method of claim 11, wherein additional antioxidants are not added to the food product.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INDUCTION TIME 2
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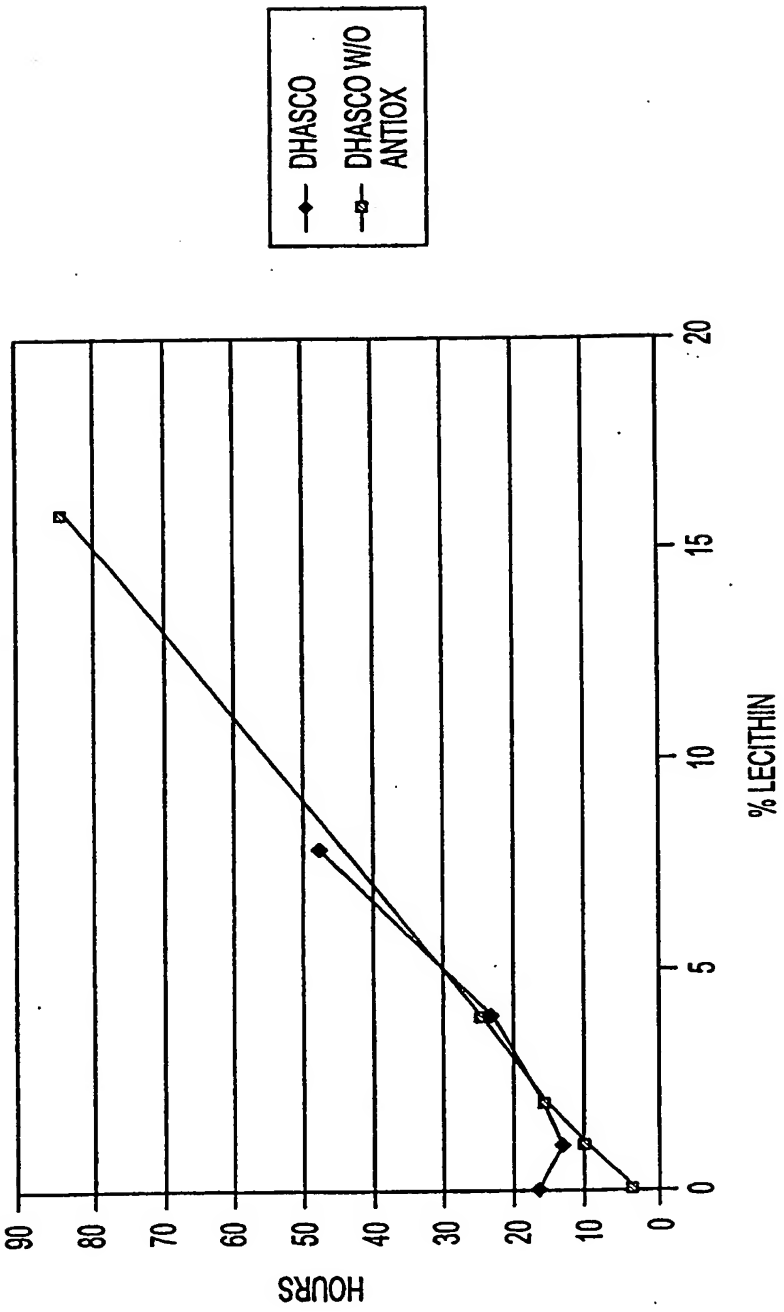
FIG. 1


DHASCO
DHASCO W/O ANTIOX
HOURS
90
80
70
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5
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FIG. 2